Polycomb repressor complex 2 function in breast cancer (Review)

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Abstract. Epigenetic modifications are important contributors to the regulation of genes within the chromatin. The polycomb repressive complex 2 (PRC2) is a multi-subunit protein complex that is involved in silencing gene expression through the trimethylation of lysine 27 at histone 3 (H3K27me3). The dysregulation of this modification has been associated with tumorigenicity through the increased repression of tumour suppressor genes via condensing DNA to reduce access to the transcription start site (TSS) within tumor suppressor gene promoters. In the present review, the core proteins of PRC2, as well as key accessory proteins, will be described. In addition, mechanisms controlling the recruitment of the PRC2 complex to H3K27 will be outlined. Finally, literature identifying the role of PRC2 in breast cancer proliferation, apoptosis and migration, including the potential roles of long non-coding RNAs and the miR-200 family will be summarized as will the potential use of the PRC2 complex as a therapeutic target.

Contents

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1. Introduction

Epigenetic modifications, including DNA methylation and histone modifications, play an important role in gene regulation. The dysregulation of these modifications can result in pathogenicity, including tumorigenicity. Research has indicated an important influence of the trimethylation modification at lysine 27 on histone H3 (H3K27me3) within chromatin. This methylation is involved in the repression of multiple genes within the genome by condensing DNA to reduce access to the transcription start site (TSS) within gene promoter sequences (1). The recruitment of H1.2, an H1 histone subtype, by the H3K27me3 modification has been a suggested as a mechanism for mediating this compaction (1). The present review discusses the function of the H3K27me3 regulator, polycomb repressive complex 2 (PRC2), and its contribution to cancer, specifically highlighting alterations in breast cancer.

2. Polycomb repressive complex 2

PRC2 is a multi-subunit protein complex that mediates the mono-, di and tri-methylation of H3K27 to silence gene expression (1-6). First established in *Drosophila*, the polycomb group (PcG) genes are well conserved across species through to human cells (7-10). The PRC2 complex contains 4 core subunits, including the enhancer of zeste homolog 1 or 2 (EZH1/2), embryonic ectoderm development (EED), suppressor of zeste 12 (SUZ12) and retinoblastoma associated protein 46/48, (RbAP46/48), also known as RBBP4/7 (Fig. 1) (9,11). This core complex can additionally interact with accessory proteins consisting of the jumonji, AT-rich interactive domain 2 (JARID2), AE binding protein 2 (AEBP2) and polycomb-like protein (PCL) (11-15). Together, these subunits can form a holo-PRC2 complex to efficiently methylate histone H3. In general, approximately 70% of H3 histones can be methylated by PRC2, indicating the main regulatory role PRC2 has within the genome (4).

3. PRC2 core subunits

Although there are 4 core subunits of PRC2, only SUZ12, EED and EZH2 are required for the basic function of this complex (16-18). Within the core complex, there are two functional lobes that govern catalytic and regulatory duties. The catalytic lobe consists of the VEFS domain in SUZ12 and the SANT1L/2L, CXC motif and suppressor of variegation,

enhancer of zeste, trithorax (SET) domains all within the EZH2 subunit (19). The methyltransferase activity of PRC2 has been specifically linked to the SET domain of EZH2 (2,3,5,20). By contrast, the regulatory lobe consists of the SR motif and SET activation loop domain (SAL) of the EED subunit (19). The SAL domain is required for catalytic function by playing a main role in the coordination of these two lobes (19,21). The interactions between these domains allow EED to recognize the presence of the H3K27me3 modification through the WD40 domain and stimulate the methyltransferase activity produced through the EZH2 subunit (17,22). Overall, the EED subunit is important for reading the modifications, SUZ12 is important for the stabilization of this interaction and EZH2 is the methyltransferase subunit. When individually isolated, these subunits do not have PRC2 methyltransferase activity, suggesting that these peptides have to cooperate to accomplish their functions (18,23).

The interaction between EED and EZH2 is facilitated through the EED binding domain (EBD) of EZH2 (24). This interaction entails the formation of a β-propeller through a 7 WD40 repeat sequence on the EED subunit to establish Van der Waals interactions and hydrogen bonds with the EBD (24). EZH2 envelops EED through multiple associations, closely linking these subunits structurally and as a result, functionally. In general, methyltransferase activity is initiated by the EED WD40 domain reading the H3K27me3 modification to induce a rotational change of the PRC2 confirmation and signals to the EZH2 subunit to activate catalytic function (19). The knockdown of EED is associated with a global reduction in H3K27me3 levels (25).

The second subunit, SUZ12, is involved in the stabilization of the PRC2 complex during catalytic activity (26). This stabilizing action is considered to be specifically linked to the VEFS domain of SUZ12 (27). More detailed research is required for the full analysis of processes involved with this subunit; however, the overall importance of this subunit is upheld by the observation that the knockdown of SUZ12 is also associated with a global reduction in H3K27me3 levels (18,26).

The third subunit, EZH1/2, is considered the writer protein in which the catalytic function of PRC2 is carried out through. This peptide is a methyltransferase that adds a methyl group to lysine 27 on histone H3 through activity within the SET domain (21,28). Both EZH1 and EZH2 have the capacity to form PRC2 complexes, but may not be completely interchangeable. EHZ2 has been observed to have a higher methyltransferase activity compared to EZH1 (29). For instance, EZH1 has been observed to specialize in only the mono- and di-methylation of H3K27, while EZH2 catalyzes mono-, di- and trimethylations (30). In addition to this complex, EZH2 may have PRC2-independent activity that will be discussed below in the present review.

Finally, some research considers RBBP4/7 part of the core subunits of PRC2, while others consider it as an accessory protein. In general, these peptides play a role in the stabilization and regulation of PRC2-chromatin interactions by binding the unmethylated histone H3K4 tail (31). This binding is impaired when H3K4me3 is present, therefore indicating to the PRC2 complex that the gene is currently active (31,32). Although only SUZ12, EED and EZH2 are required for any enzymatic activity, RBBP4/7 may be considered a core subunit due to the control it contributes to the complex (18,23,33). Furthermore, RBBP4 and RBBP7 are part of the WD40 repeat protein family that acts to stabilize the PRC2-chromatin interaction. This occurs through the simultaneous binding of PRC2, H3 and H4 (31,34,35). The binding of H4 by RBBP4/7 causes a structural change to unfold helix 1 of H4 to support this process (34). Within the PRC2 complex, RBBP7 is closely associated with SUZ12, but only weakly to EED (16). Moreover, this incorporation into the PRC2 complex is SUZ12-dependent (16). Functionally, RBBP4 and RBBP7 are similar and have been studied closely together.

4. PRC2 accessory proteins

The accessory proteins interact with the core subunits ultimately to enhance PRC2 function by increasing stability, regulating catalytic function or aiding in the recruitment of the PRC2 complex to a locus. Accessory proteins that interact with the PRC2 complex include JARID2, AEBP2 and PCLs.

JARID2 contains 4 domains, 2 jumonji and 2 DNA binding (14,36). This peptide occupies the same area of the chromatin as PRC2 with affinity to 90% of the previously mapped PcG genes, supporting the hypothesis that JARID2 is a PRC2 accessory protein (15,33). Furthermore, the DNA binding domains contain zinc fingers that interact with nucleosomes to recruit and stabilize both polycomb complexes involved in epigenetic gene silencing, PRC1 and PRC2 (14,33,37,38). JARID2 has also been observed to interact with mono-ubiquitinated H2A lysine 119, a modification established by PRC1, to allow PRC2 and PRC1 to crosstalk (39-41). This may be a mechanism supporting the interdependency of these two polycomb complexes during epigenetic gene repression. Moreover, JARID2 is considered a regulator of PRC2-mediated H3K27me3 based on methylation levels already present (12). While JARID2 has been observed to recruit PRC2 to a locus, the jumonji domains act to inhibit methyltransferase activity (15,42). This domain negatively regulates the catalytic function of PRC2 to act as a controller (15,42). In general, JARID2 is not required for maintaining global H3K27 methylation; however, it assists this process.

Closely associated with JARID2 is AEBP2 (41). Together, these proteins stimulate PRC2 methyltransferase activity in a synergistic manner (38). AEBP2 has 2 isoforms, short and long, which are developmental stage-specific (13). The short isoform is associated with the embryo developmental stage, while the large isoform is associated with the adult stage (13). This is a zinc finger protein that interacts with both PRC2 and DNA, mediating PRC2 recruitment and stabilization (13). Target loci of AEBP2 have been mapped close to those of PRC2, suggesting that this is a targeting protein for PRC2 (13). In general, AEBP2 is required for optimal PRC2 function (16).

Finally, PCL is a group of peptides that contain Tudor domains that can bind H3K36me3 markers, known to increase PRC2 recruitment (43,44). Recruitment processes will be discussed in detail in the following section. Overall, there are 3 mammalian PCLs important for PRC2 activity. These include PHF1, MTF2 and PHF19, also known as PCL1, PCL2 and PCL3, respectively (12,45). They all contain Tudor domains,

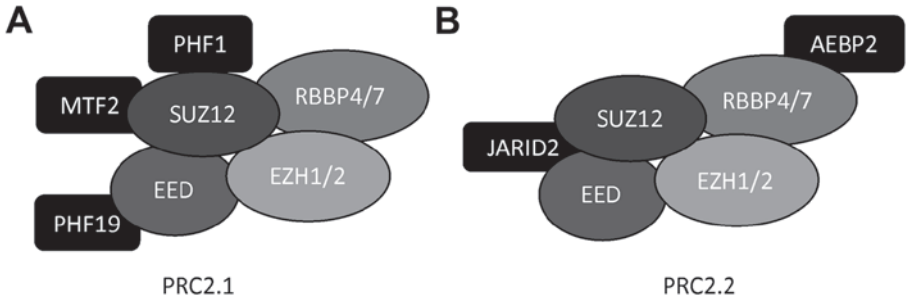

Figure 1. (A and B) The PRC2 complex consists of 4 core subunits, including EZH1/2, EED, SUZ12 and retinoblastoma associated protein 46/48, (RbAP46/48 or RBBP4/7). RBBP4/7 is closely associated with SUZ12, but not with EED. In addition to the core subunits the PRC2 complex can include accessory proteins. (A) The PRC2.1 complex consists of the 4 core subunits and PCLs, including PHF1, MTF2 and PHF19, also known as PCL1, PCL2 and PCL3, respectively. (B) The PRC2.2 complex consists of the 4 PRC2 core subunits and the two accessory proteins, JARID2 and AEBP2. During recruitment, JARID2 specifically associates with the EED subunit. PRC2, polycomb repressive complex 2; EZH1/2, enhancer of zeste homolog 1 or 2; EED, embryonic ectoderm development; SUZ12, suppressor of zeste 12; PCLs, polycomb like-proteins; JARID2, jumonji, AT-rich interactive domain 2; AEBP2, AE binding protein 2.

2 plant homeodomain fingers, an extended homologous region N terminal cluster and C terminal Chromo-like domain (46). These proteins may be particularly associated with promoting new PcG modifications of genes not previously silenced. More recently, PHF20L1, another TUDOR domain containing protein has been identified as a H3K27me2 reader that can recruit PRC2 (47).

The mapping of the PRC2 complex has revealed that these accessory proteins form 2 distinct complexes, identified as PRC2.1 and PRC2.2 (Fig. 1) (12). PRC2.1 was identified as containing PCLs as the accessory proteins, while PRC2.2 exclusively contained JARID2 and AEBP2 (Fig. 1) (12). Further analysis suggested that PRC2.1 was involved in the *de novo* recruitment of PRC2 to CpG islands that lack H3K27me3 (46,48). By contrast, PRC2.2 has been suggested to be involved in methyltransferase activity through recruitment to chromatin that has PcG-dependent modifications, H3K119ub or H3K27me3, already present (39,41).

5. Recruitment of PRC2

Once H3K27 is trimethylated, the histone must remain in this state to continue the repression of the gene at hand, avoiding improper transcription. When an H3K27me3 is already present, PRC2 can be recruited to the chromatin, allowing EED to read the marker (Fig. 2A) (22,49,50). A positive feedback loop can then be initiated to stimulate PRC2 methyltransferase activity. This activation can propagate the H3K27me2/3 marker in *cis* and far *cis* through long-range contacts (50). In general, this method of recruitment is involved in the maintenance and propagation of H3K27me3, although this is not the sole form of recruitment.

PRC1, another PcG complex, also plays a significant role during the recruitment of PRC2. PRC1 and PRC2 have an interdependent association where they can recruit one another. This is supported by the co-localization of PRC1 and PRC2 to approximately 10-15% of all genes (51-54). PRC1 is an ubiquitin ligase that can be recruited to H3K27me3 by chromobox subunits (CBX), CBX7 and CBX8 (55,56). Subsequently, PRC1 recruitment and activation initiate the ubiquitination of lysine 119 at histone 2A (H2AK119ub1) (40,41). As a result, the ubiquitination marker can recruit and activate PRC2, again spreading H3K27 trimethylation (Fig. 2B) (41,57,58). TRIM37 has also been observed to ubiquitinate H2AK119 (59). This protein binds both PRC1 and PRC2, specifically targeting genes for silencing (59). Although the mechanism of PRC2 recruitment by TRIM37 has not yet been fully established, the knockdown of TRIM37 induces the re-expression of silenced genes due to the dissociation of PRC1 and PRC2 from the target locus (59).

Overall, recruitment to sites with H3K27me3 or H2AK119ub already present occurs through JARID2 (39,41). This is largely a PRC1-dependent process in which JARID2 can dock on DNA by interacting with the H3K119ub modification (39,41). JARID2 then catalytically stimulates the PRC2 complex through the methylation of JARID2 at lysine 116 (Fig. 2B) (60). The function of this modification is to allow JARID2 to bind EED at the WD40 domain, inducing a conformational change and leading to the increased catalytic activity of EZH2 (60). Additional interactions of other proteins within the holo-protein complex help stabilize the PRC2 interaction with the chromatin. This includes JARID2, AEBP2, RBBP4/7 with histone H3 or H4, EED and SUZ12.

Without H3K27me3, the recruitment of PRC2 occurs through the presence of different accessory proteins. While PRC2.2, including JARID2 and AEBP2 can be recruited effectively through H3K27me3 or H2AK119ub markers, *de novo* recruitment appears to be limited to the PRC2.1 complex containing PCLs (46,48). This recruitment appears to occur at CpG islands (CGI), an area of DNA that has a high frequency of cytosine and guanine (Fig. 2C) (46,48). This area may have fewer nucleosomes; however, this observation is not a direct predictor of recruitment (61). More specifically, polycomb response elements (PREs) within these CGI are suggested to recruit PRC2 and PRC1 (62). These have been well characterized in *Drosophila*; however, identification and characterization within mammals have yet to be confirmed (63). More distinguished within mammalian cells is the *de novo* recruitment of PRC2 through the H3K36me3 modification (Fig. 2C). H3K4me3 in combination with the H3K36me3 modification is associated with active genes (64). PRC2 may use the H3K36me3 modification to create balance within the chromatin, initiating the silencing of genes that were previously active. PCL proteins are specifically involved in this *de novo* recruitment. PHF19 can interact with H3K36me3 through its Tudor domain to create a contact

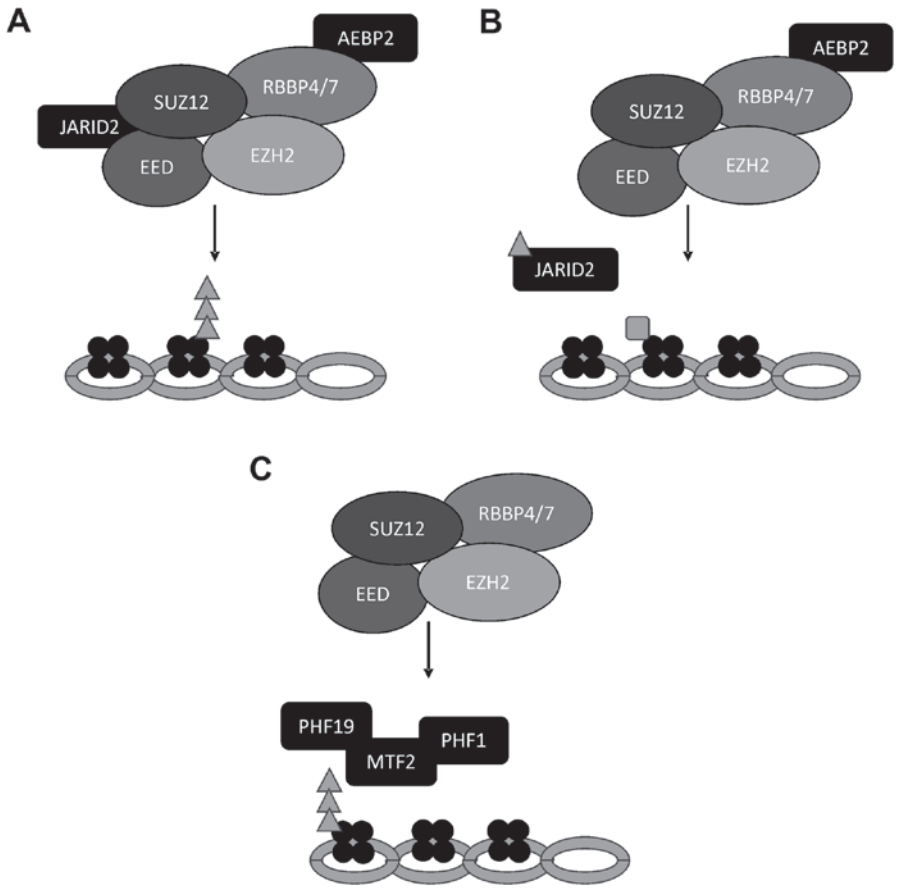

Figure 2. The possible mechanisms of recruiting the PRC2 complex to initiate the trimethylation of lysine 27 at histone H3 (H3K27me3). (A) PRC2 can be recruited to H3K27me3 (grey triangles) and recognized by the EED subunit. This activates EZH2 methyltransferase activity, creating a positive feedback-loop through EED to propagate H3K27me3 modifications within the chromatin. (B) PRC1-mediated ubiquitination (grey square) of lysine 119 at H2A (H2AK119ub) is recognized by JARID2 inducing methylation at lysine 116 (grey triangle). This methylation interacts with the WD40 domain on the EED subunit to initiate recruitment and methyltransferase activity. (C) Polycomb-independent recruitment occurs through PCL recognition of the trimethylation of lysine 36 on histone H3 (H3K36me3) at CpG islands. PHF19 can interact with H3K36me3 (grey triangles), while MTF2 and PHF1 can bind CpG islands to recruit PRC2 to the chromatin. PRC, polycomb repressive complex; EZH2 enhancer of zeste homolog 2; JARID2, jumonji, AT-rich interactive domain 2; EED, embryonic ectoderm development; SUZ12, suppressor of zeste 12.

point to recruit PRC2 (Fig. 2C) (43,65,66). Additionally, the demethylase, NO66, is recruited through PHF19 interactions with H3K36me3, overall resulting the gain of H3K27me3 and the loss of methylation at H3K36 by NO66 activity (65). MTF2 has also been observed to bind CGIs in the presence of H3K36me3 and is an essential recruiter for PRC2 through *de novo* mechanisms in both mice and *in vitro* models (46,48). Both MTF2 and PHF1 are observed to fold into a winged helix to successfully bind unmethylated CpG motifs (Fig. 2C) (46). Once PRC2 is successfully recruited, a positive feedback loop through the EED subunit sustains the methyltransferase activity at the CGIs to promote and propagate H3K27me3 (22).

Finally, long non-coding RNAs (lncRNAs) may additionally contribute to the recruitment of PRC2. A lncRNA termed HOX transcript antisense RNA (*HOTAIR*) has been identified to promote PRC2 binding in *trans* (67,68). The *HOTAIR*-PRC2 mechanism seems to regulate specific genes, including *HOXD10*, *PCDH10*, *PCDHBS*, *APC2* and *NLK* (69,70). *HOTAIR* provides scaffolding for histone modification enzymes including PRC2 and LSD1, a lysine 4 demethylase (68). The binding of these enzymes occurs at the 5' and 3' ends of *HOTAIR*, respectively (68). Alterations to *HOTAIR* levels have been observed in breast cancers, which will be addressed in detail below.

6. Effect of PRC2 on breast cancer

The altered regulation of genes is an important contributor to the pathogenicity of cancer. Since PRC2 alters epigenetic markers to repress genes, this complex may act to silence tumor suppressors, advancing tumorigenesis. Due to PRC2 targeting multiple genes, pin-pointing individual genes being silenced that directly contribute to cancer progression is a complex task. Some highlighted alterations in breast cancer that research has discovered are described below; however, it is unlikely this is the entirety of the effects of this complex.

PRC2 increases breast cancer cell proliferation. The proliferation of tumor cells is reduced when PRC2 subunits are knocked down, suggesting that this complex plays a role in breast tumor growth (20,71). Specific genes linked to this process have yet to be identified within breast cancer cells, but are overall linked to *EZH2* overexpression (20,72,73). The levels of positive regulators of the cell cycle, including cyclinD1, cyclinE1, cyclinA2 and cyclinB1 are significantly

reduced in cells that lack *EZH2* expression (71). EZH2, as well as EED and SUZ12 are downstream targets of the pRB-E2F pathway, in that E2F can regulate the expression of these PCR2 subunits (71). Additionally, EED has also been identified as critical for proliferation, as the inhibition of EED-EZH2 interaction impairs tumor cell proliferation, thus providing further evidence that PRC2 activity facilitates proliferation (71,74).

PRC2 increases breast cancer cell invasion and metastasis. Metastatic breast cancer cells within adjacent lymph nodes have been shown to overexpress PRC2, which could suggest cells have acquired this alteration to gain the capacity to successfully metastasize (75). Within the primary tumor, matrix metallopeptidases (MMPs) play a key role in the degradation of the extracellular matrix (ECM) in the tumor microenvironment (TME) (76). The degradation of the ECM results in a higher frequency of the invasion and metastasis of breast cancer (77). Elevated EZH2 levels are associated with the repression of tissue inhibitors metalloproteinases (TIMPs) in triple-negative breast cancer (TNBC) (78). Specifically, EZH2 represses *TIMP* expression through the induction of the H3K27me3 silencing modification (78). Without TIMP proteins, MMP2 and MMP9 activity is increased, thus potentiating the metastatic capacity of breast cancer cells (78). This has also been observed in other types of cancer, such as ovarian and prostate cancer (79,80).

Another potential target of the PRC2 complex that affects metastatic potential is the repression of growth differentiation factor-15 (GDF15) (81). Yes-associated protein (YAP) is an upstream regulator of the PRC2 complex that promotes trimethylation of H3K27 at the *GDF15* promoter, leading to the suppression of *GDF15* (81). When YAP is knocked down, the metastatic potential is decreased, suggesting that this recruitment of PRC2 to *GDF15* may affect the aggressiveness of breast cancer cells (81). *YAP* expression and its role in breast cancers have been controversial with some research indicating a tumor-suppressive role, while others suggesting an oncogenic role (81-84).

PRC2 decreases apoptosis. Compounds that suppress PRC2 activity, such as 3-deazaneplanocin A (DZNeP), have been observed to re-induce apoptosis, leading to targeted cell death (85). F-box protein 32 (FBXO32) has been identified as a key effector of apoptosis in this process (85). Due to this re-expression, it can be suggested that during abnormal PRC2 activity, apoptosis is suppressed by reducing FBXO32 levels. Further support for a role of PRC2 in apoptosis includes the observation that elevated levels of EZH2 are associated with reduced apoptosis following DNA damage, while decreased levels of EZH2 potentially facilitate apoptosis (86-89). Mechanistically, FBXO32 is repressed by EZH2 (86). FBXO32 normally directly binds p21 for protease degradation within p53-proficient cells and additionally induces apoptosis in p53-deficient cells through CHK1 activation (86). When EZH2 is depleted, G1 and G2/M checkpoints of the cell cycle are inhibited, leading to the initiation of apoptosis with or without p53 involvement (86).

Another suggested pathway for evading apoptosis involves the deregulation of the pRB/E2F pathway. When EZH2 is activated by E2F1, E2F1-mediated apoptosis is suppressed (87). This regulation of EZH2 leads to methyltransferase activity at the promoter of *BIM1* to induce the trimethylation of H3K27, and furthermore, to suppress *BIM*1 expression (87). This process, induced by the pRB/E2F pathway, suggests that suppressing *BIM1* is a potential alteration that occurs in cancer cells to escape apoptosis through epigenetic regulations. Within clinical cases, a high *EZH2* expression is associated with a poor outcome, while *BMI1* overexpression is associated with good outcomes (90).

PRC2 induces epithelial-to-mesenchymal transition (EMT). An indication of EMT is the loss of E-cadherin (*CDH1*) expression, a cell-to-cell adhesion molecule present in epithelial cells (91). The downregulation of *CDH1* is associated with increased invasiveness and progression in breast cancer (91-93). The repression of *CDH1* has been suggested to occur through the H3K27me3 marker created by the PRC2 complex (94). SNAI1 is associated with the increased binding of PRC2 to the promoter of *CDH1*, leading to increased metastasis, invasion and EMT (94). *SNAI1* can be regulated by the microRNA (miRNA/miR)-200 family to induce this PRC2 recruitment. This will be addressed in further detail below.

7. EZH2 has oncogenic properties independent of the PRC2 complex

Although EZH2 is a core PRC2 protein, it may also function independent of the PRC2 complex (95,96). It has been suggested that EZH2 forms an additional complex with foxhead box M1 (FOXM1) under hypoxic conditions (97). Hypoxia-inducible factor 1-alpha (HIF1A) induces *EZH2* expression, but suppresses *SUZ12* and *EED* expression and this scenario of elevated EZH2 protein levels in the absence of SUZ12 and EED promotes EZH2-FOXM1 interaction, resulting in the elevated expression of MMPs and increases tumor invasion (97). With this in mind, research that aims to address the effects of the PRC2 complex on cancer progression cannot solely measure EZH2 activity and increased H3K27me3 levels should be measured to confirm PRC2 activity.

8. Dysregulation of long non-coding RNAs alters PRC2 activity in cancer

lncRNAs have been emphasized more recently as regulators of PRC2 activity. The long non-coding RNA termed *HOTAIR* has been observed to promote PRC2 binding by acting as a scaffold (68). The dysregulation of this lncRNA has been observed during the progression of breast cancer and is associated with poorer clinical outcomes (98,99). Particularly, the increased expression of *HOTAIR* is observed in primary breast cancers and metastases, and predicts the detachment of tumor cells, potentially leading to metastasis (69). By contrast, the knockdown of *HOTAIR* represses migration, invasion and metastasis within *in vitro* and *in vivo* models (69). Another HOX transcript antisense RNA termed *HOTAIRM1* has recently been implicated in promoting tamoxifen resistance in breast cancer through inhibition of PRC2 activity (100).

Additionally, lncRNA *NBAT1* interacts with the EZH2 subunit and may also be dysregulated in breast cancer. *NBAT1* has been identified to be downregulated in breast cancer cells, and has been shown to be associated with metastatic lesions and poor clinical outcomes (101). *In vitro*, *NBAT1* expression

inhibits migration and invasion through the activation of DKK1, an inhibitor of the WNT signalling pathway (101). The overexpression of *NBAT1* downregulates H3K27me3 at the *DKK1* promoter, while the lack of *NBAT1* increases H3K27me3 at the *DKK1* promoter, suppressing the expression of this gene (101). Therefore, *NBAT1* has the capacity to negatively regulate PRC2 activity at the *DKK1* promoter, possibly contributing to the pathogenicity of cancer.

A high expression of the lncRNA, *LINC00511*, has been shown to be associated with a poor prognosis of patients with breast cancer. *LINC00511* has been shown to interact with EZH2 and promote recruitment of the PRC2 complex to the promoter of the cell cycle inhibitor *CDKN1B* in estrogen receptor-negative breast cancer (102).

Finally, higher levels of the lncRNA *DANCR* have been found to be associated with a poor clinical outcome of patients with TNBC (103). *DANCR* and EZH2 act synergistically to repress the TIMP2/3 and in turn, increase MMP activity (104). The knockdown of *DANCR* suppresses the invasion and migration of TNBC and prostate cancer cells (103,104).

9. The miR-200 family and PRC2 activity in breast cancer

The miR-200 family is a family of small non-coding RNAs consisting of miR-200a, miR-200b, miR-200c. miR-141 and miR-429 that are expressed as two clusters, the miR-200c/141 and miR-200b/200a/429 cluster (105-107). miRNAs are involved in the post-transcriptional regulation of genes and inhibit the translation of target genes (108-110). Research from the authors' laboratory and those of other researchers has indicated that miR-200s are expressed at high levels in luminal breast cancer and luminal breast cancer cell lines; however, their expression is significantly reduced in TNBC and TNBC cell lines (111-117). The miR-200 family negatively regulates the expression of genes involved in EMT, such as *TWIST1/2*, *ZEB1/2* and *SNAI1/2* (108,118-120). Additionally, *SUZ12* may also be an important target of the miR-200 family. SUZ12 translation is directly inhibited by miR-200b (121,122) and thus modifications resulting in the reduced expression of miR-200b in breast cancer cells promote elevated SUZ12 levels and increased H3K27me3 levels by PRC2. Increased PRC2 activity and the elevated expression of EMT-related genes, such as *ZEB1* ensure that *CDH1* transcription is repressed and maintains breast cancer cells in a mesenchymal phenotype (92,123). Of note, miR-200s can also be regulated by PRC2. The miR-200b/a/429 cluster can be silenced through PRC2-mediated histone H3K27me3 modification (124,125). Therefore, feedback loops between miR-200 expression and PRC2 activity may exist in cells.

10. Therapeutics targeting PRC2 activity in cancers

PRC2 has been identified as a potential target for the treatment of breast cancer. DZNep can effectively reactivate genes that have been silenced by PRC2 in breast cancer (85). This drug functions to inhibit the methyl group donor, S-adenosylhomocysteine hydrolase, and results in the degradation of EZH2, leading to a decrease in H3K27 di and tri-methylation in the promoters of tumor suppressor genes (126,127). DZNep also appears to promote apoptosis by increasing the expression of apoptosis-related genes, such as *FBXO32*, *TGFBI*, *IGFBP3* and *PPPIR15A* (85,126). It should be noted that DZNep is not a specific EZH2 inhibitor and thus, other intracellular targets may also mediate the effects of DZNep (127). More recently, MS1943 (1) has been described as a EZH2 selective degrader that is cytotoxic to multiple TNBC cell lines *in vitro* and in xenograft models (128).

Tazemetostat is an EZH2 inhibitor which functions competitively to inhibit the co-factor, S-adenosyl-L-methionine (SAM), an essential component for EZH2 activity (129,130). Tazemetostat recently gained FDA approval for epithelial sarcomas and clinical trials are currently underway in other types of cancer (131). Other EZH2 inhibitors that target SAM are in early-stage clinical trials, including GSK2816126 and CPI1205 (129) (NCT02082977, NCT02395601, NCT03525795 and NCT03480646).

In addition to targeting the EZH2 subunit of the PRC2 complex, other PRC2 components are also being investigated as therapeutic targets. The EED-EZH2 interaction is critical for EZH2 function and stabilized alpha-helix of EZH2 (SAH-EZH2) peptides have been developed to disrupt the EED-EZH2 interaction, which results in decreased H3K27me3 levels and the degradation of EZH2 (132).

As research uncovers the mechanisms that regulate H3K27me3, additional targets outside of the core PRC2 proteins have been identified as possible targets. As mentioned previously, the long non-coding RNA *HOTAIR* has been observed to recruit PRC2 to target genes (68). The small molecule AC1Q3QWB (AQB) has been identified to selectively disrupt the interaction of *HOTAIR* with EZH2 during PRC2 recruitment, resulting in elevated levels of *HOTAIR* target genes such as APC2 (133). Elevated APC2 levels result in the degradation of β-catenin and the suppression of pro-tumorigenic Wnt/β-catenin signaling (133). Other research has aimed to disrupt *HOTAIR*-EZH2 interaction using peptide nucleic acids (PNAs) that bind to single-stranded regions of *HOTAIR* (134). Treatment with PNAs has been shown to result in less invasion, reduced tumor formation and increased chemosensitivity in breast cancer cells, as well as ovarian cancer cells (134). Another long non-coding RNA, named *DANCR* has also been targeted. The nanoparticle-mediated RNA inhibition of *DANCR* has been shown to knockdown *DANCR* 80-90% in cells up to 7 days (135). This administration at a cellular level has been shown to be associated with the downregulation of PRC2 methyltransferase activity at H3K27me3, as well as the reduced Wnt/EMT signaling, migration and proliferation of TNBC cells (135).

Therapies designed to target PRC2 may enhance the efficacy of existing therapeutic strategies. For example, PRC2 decreases accessibility to regions of the DNA through chromatin compaction. Chromatin compaction can decrease the sensitivity of cancer cells to chemotherapeutic agents, such as anthracycline (136). In addition, the use of EZH2 inhibitors to decrease global H3K27me3 may enhance the efficacy of monoclonal antibodies targeting HER2 (137). *De novo* resistance in patients has been associated with elevated levels of global H3K27me3 levels in $HER2^+$ breast cancer and the administration of EZH2 inhibitor GSK126 in combination with an anti-ErbB2 monoclonal antibody has been shown to significantly suppress tumor growth in an orthotopic mouse tumor model (137).

11. Conclusion

In summary, increased global levels of H3K27me3 can be associated with breast cancer progression, including proliferation, invasion, metastasis, evading apoptosis and EMT. This modification involves the dysregulation of the PRC2 multi-subunit protein which, as stated, may occur through mechanisms involving altered levels of the miR-200 family or lncRNAs. Finally, recent studies utilizing the overexpression of PRC2 as a possible mechanism of pathogenicity have seen some promise in targeting this protein complex. Advancing the understanding of this complex and its potential role in tumorigenicity may provide novel breast cancer therapies.

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Authors' contributions

CJM wrote the manuscript and RAM edited the manuscript. Both authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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